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Co-Therapy of Silymarin and Vitamin E Attenuates Doxorubicin-Induced Hepatotoxicity in Rats by Mitigating Oxidative Damage and Preserving Hepatic Architecture.

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Abstract

Background: Doxorubicin (DOX) is one of the most potent anthracycline antibiotics that is widely used in oncology. Its clinical use is greatly limited by dose-dependent toxicities, including hepatotoxicity which is caused by oxidative stress and inflammation.

Objective: This study investigated whether the combining of silymarin (a polyphenolic flavonolignan) and vitamin E (a lipophilic membrane antioxidant) had exerted hepatoprotective effects against DOX-induced liver injury in rats.

Methods: 48 male Wistar rats were assigned into six groups, including a negative control, a DOX control (15 mg/kg cumulative intraperitoneal dose), and treatment groups receiving silymarin 200 mg/kg, vitamin E 200 mg/kg, or their combination, and recovery group. Liver injury was assessed by measuring tissue biomarkers for oxidative stress (MDA, SOD), inflammation (IL-6), and apoptosis (Caspase-3, Bax, Bcl-2) and serum biomarkers (AST, ALT, ALP, TSB), while histological analysis was used to assess necrosis, fatty degeneration, fibrosis, and inflammatory cell infiltration.

Results: In this study DOX administration caused severe liver injury, evidenced by significant increase in hepatic MDA, IL-6, and Caspase-3, alongside depleted SOD and Bcl-2 levels. The combination therapy restored oxidative, inflammatory, and apoptotic markers close to the normal levels more effectively than monotherapy.

Conclusion: The administration of silymarin and vitamin E concurrently offers greater protection against DOX-induced hepatotoxicity through a synergistic mechanism involving antioxidant regeneration and membrane stabilization.

العلاج المشترك بالسيليمارين وفيتامين "هـ" يخفف من سُميّة الكبد الناجمة عن الدوكسوروبيسين في الجرذان من خلال الحد من التلف التأكسدي والحفاظ على البنية الكبدية

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الخلاصة

يُعد الدوكسوروبيسين DOX أحد أقوى المضادات الحيوية من فئة الأنثراسيكلين المستخدمة على نطاق واسع في علاج الأورام. ومع ذلك، فإن استخدامه السريري مقيد بشكل كبير بسبب حالات التسمم المعتمدة على الجرعة، ومنها التسمم الكبدي الناتج عن الإجهاد التأكسدي والالتهاب. بحثت هذه الدراسة ما إذا كان الجمع بين السيليمارين وهو فلافونوليجنان متعدد الفينول وفيتامين هـ وهو مضاد أكسدة محب للدهون لغشاء الخلية يحقق تأثيرات تآزرية واقية للكبد ضد إصابات الكبد المستحدثة بالدوكسوروبيسين في الجرذان. تم تقسيم ٤٨ من ذكور جرذان ويستار إلى ست مجموعات متساوية، شملت: مجموعة السيطرة السالبة، ومجموعة السيطرة للدوكسوروبيسين التي تلقت جرعة تراكمية ١٥ ملغم/كغم داخل الصفاق، ومجموعات علاجية تلقت السيليمارين بجرعة ٢٠٠ ملغم/كغم، أو فيتامين هـ بجرعة ٢٠٠ ملغم/كغم، أو مزيجاً منهما معاً، ومجموعة التعافي الطبيعي. تم تقييم إصابة الكبد عبر قياس المؤشرات الحيوية النسيجية للإجهاد التأكسدي مثل MDA وSOD، ومؤشرات الالتهاب مثل IL-٦، ومؤشرات موت الخلايا المبرمج مثل Caspase-٣ وBax وBcl-٢، بالإضافة إلى مؤشرات مصل الدم وتشمل AST وALT وALP وTSB. كما استُخدم الفحص النسيجي المرضي لتقييم النخر، والتنكس الدهني، والتليف، وتسلل الخلايا الالتهابية. أدى إعطاء الدوكسوروبيسين في هذه الدراسة إلى إحداث إصابة كبدية شديدة، تمثلت في زيادة معنوية واضحة في مستويات MDA وIL-٦ وCaspase-٣ وBax في أنسجة الكبد، يصاحبها انخفاض ملحوظ في مستويات SOD وBcl-٢. وفي المقابل، أظهر العلاج المشترك بالسيليمارين مع فيتامين هـ قدرة فائقة على استعادة المؤشرات التأكسدية والالتهابية ومؤشرات الموت الخلوي وإعادتها لتكون قريبة من المستويات الطبيعية بكفاءة أعلى من العلاج الأحادي. يتبين أن الإعطاء المتزامن للسيليمارين وفيتامين هـ يمنح حماية كبدية أقوى وأشمل ضد التسمم الكبدي المستحدث بالدوكسوروبيسين، وذلك من خلال آلية تآزرية تعتمد على تجديد مضادات الأكسدة وتثبيت الأغشية الخلوية.

الكلمات المفتاحية: سيليمارين؛ فيتامين هـ؛ دوكسوروبيسين؛ التسمم الكبدي؛ واقي للكبد.

INTRODUCTION

Doxorubicin (DOX), an anthracycline antibiotic that originated from Streptomyces peucetius in the 1960s [(1)]. It is considered a standard medication in modern oncology [(2)]. DOX is very effective against acute leukemias, lymphomas, breast carcinoma, and many solid tumors [(3)]. Its mechanism of action includes intercalating into the DNA double helix. It inhibits the synthesis of topoisomerase II (TOP2A) that leads to double-strand breakage [(4, 5)]. However, DOX undergoes redox cycling and Iron-Mediated reaction generating reactive oxygen species (ROS) that cause oxidative damage to lipids, DNA, and cell membranes that leads to dose-limiting side effects [(6)].

In addition to the dose-limiting cardiotoxicity, the liver is also affected by doxorubicin and making the liver responsible for many metabolic problems that will create a second layer of toxicity because the liver is the main site for DOX metabolism [(7)]. Here, the liver produces a paradox effect when doxorubicin is administered. The liver attempts to metabolize the drug, but actually it amplifies the doxorubicin toxicity. The liver uses Carbonyl Reductase enzyme for doxorubicin detoxification [(8)]. This enzyme converts doxorubicin to the semiquinone radical directly within hepatocytes, ultimately leading to the generation of a localized storm of superoxide anions that depletes hepatic glutathione storage and causes hepatic injury [(9)]. This leads to prolonged retention of the drug and its toxic metabolite, doxorubicinol, in the circulation, which in turn intensifies the oxidative attack on the heart and other organs [(10)]. So, the outcome of liver injury is not just a local organ dysfunction but an amplification of the overall systemic toxicity profile. In addition to that, the accumulation of doxorubicin inhibits the expression of CYP450 isoenzymes (specifically CYP2C and CYP3A) [(11)]. This creates another problem that not only damages the liver but also impairs its ability to clear other co-administered drugs and leads to increase the risk of developing drug-drug interactions which put another burden during the treatment course of malignancies [(12,13)].

Antioxidant treatments protect against these life-threatening side effects. Silymarin, a purified extract derived from milk thistle (Silybum marianum). It is a potent hepatoprotective agent composed of flavonolignans. Silymarin is best known as a hepatoprotective agent. Its protective action involves:

Membrane stabilization: It binds to receptors on the hepatocyte cell membrane altering physical

properties so that toxins cannot enter [14].

Regeneration: It stimulates protein synthesis by interacting with RNA polymerase I in the nucleus, therefore accelerating the regeneration of damaged liver tissue; and Anti-inflammatory action: It inhibits the NF-kappaB pathway and decreases the release of pro-inflammatory cytokines like TNF-alpha and IL-6 from Kupffer cells. This effect can help ultimately in the reduction of hepatic inflammation and necrosis [15].

While vitamin E (alpha-tocopherol) acts as the body's major lipophilic antioxidant, its protective action involves:

Lipid membrane protection: Vitamin E acts as a chain-breaking antioxidant. It incorporates within the phospholipid bilayer of both plasma and organelle membranes. When lipid peroxidation starts through reactive oxygen species (ROS), vitamin E quickly donates a hydrogen atom via hydroxyl group on its chromanol ring to propagating lipid peroxyl radical (LOO•) [16,17]. This reaction neutralizes the radical by forming stable lipid hydroperoxide plus relatively unreactive tocopheryl radical thereby effectively terminating further propagation of the chain reaction of lipid peroxidation before any substantial damage to membranes occurs [18].

Mitochondrial integrity: Vitamin E plays an important role in the maintenance of mitochondrial integrity because it protects cardiolipin and other phospholipids from oxidation. It incorporates into the mitochondrial membranes [19]. This will prevent loss of mitochondrial membrane potential, and thus it inhibits the opening of mPTP. Therefore, pro-apoptotic factors such as cytochrome c cannot leak into cytosol; blockage will happen on this intrinsic pathway of apoptosis [20].

Current research highlights the need for safer therapeutic approaches [21]. This study hypothesizes that the combined use of silymarin and vitamin E have a synergistic protective effect [22]. Theoretically, silymarin acts as an antioxidant, and it can also restore oxidized vitamin E [23]. The co-administration of both agents sustain cellular glutathione levels and offer broader protection against DOX-induced oxidative stress than either agent alone.

METHODS

Chemicals and Reagents

Doxorubicin HCl was purchased from Deva Holding A.S. (Istanbul, Turkey) . Silymarin and vitamin E (alpha-tocopherol) provided from Metabolic Maintenance (Erlanger, USA) and Adrien Gagnon (La Prairie, QC, Canada), respectively. Biochemical kits were obtained from Biolabo (Maizy, France), and tissue ELISA kits by Sunlong Biotech (Hangzhou, China).

Drug Preparation

Silymarin: Due to its poor solubility in water. It was prepared in a suspension by using 0.5% (w/v) carboxymethyl cellulose sodium as a vehicle. The suspension was prepared every day by mixing an amount of powder with a small volume of CMC-Na solution to make a smooth paste which will then be gradually diluted to final volume [24].

Vitamin E: Like silymarin, vitamin E was suspended in 1.5% hydroxypropyl methylcellulose HPMC because this agent helps stabilize and increase the bioavailability of the vitamin. This process started with adding vitamin E into HPMC solution under continuous mixing until achieving homogeneity [25].

Experimental Animals and Ethical Approval

48 adult male Wistar rats weighing 150-200g were maintained under controlled laboratory conditions (22±2 °C, 12 h light/dark cycle) and were allowed ad libitum consumption of standard chow and water. Only male rats were selected for this study to eliminate the potential confounding variables associated with the female estrous cycle. Specifically, estrogen possesses known endogenous antioxidant and hepatoprotective properties that could interfere with the assessment of doxorubicin-induced oxidative stress and the true protective efficacy of the administered antioxidants. This study was approved by the Medical Research Ethics Committee, College of Medicine, University of Mosul with reference no: UOM/COM/MREC/25-26/OCT8 dated on 26/10/2025. Ethical approval was obtained because all procedures strictly followed institutional guidelines for ethical care and use of laboratory animals. To minimize suffering, all animals were deeply anesthetized using a mixture of Ketamine (80 mg/kg) and Xylazine (10 mg/kg) administered intraperitoneally. Following the confirmation of deep anesthesia, euthanasia was humanely performed via cervical dislocation. Animals were group-housed to provide social enrichment.

Experimental Design

This prospective experimental study started following a one-week acclimatization period for the rats. They were randomly allocated into six equal groups consisting of eight rats each (n=8) using a computer-generated random number sequence to ensure simple randomization without bias. It was determined by using a power analysis designed to achieve 80% statistical power at a 5% significance level, based on expected standard deviations in primary biochemical markers. Pre-specified exclusion criteria were established prior to the experiment. Animals were to be excluded from the final statistical analysis if they experienced mortality due to technical errors during drug administration (such as unintended gastrointestinal perforation during intraperitoneal injection) or if they exhibited severe, unexpected distress unrelated to the targeted experimental pathology. The experiment was conducted over a period of six weeks (Weeks 1-6) according to specific protocols for each group as follows:

Group A (Negative Control): The healthy baseline group received daily oral vehicle solution consisting of 0.5% CMC-Na,1.5% HPMC to match the treatment groups and normal saline was given intraperitoneally in place of doxorubicin.

Group B (Doxorubicin Control): Rats in this group were treated with doxorubicin (DOX) at a dose of 5 mg/kg via the intraperitoneal route once weekly for three consecutive weeks to induce toxicity, which makes up a total cumulative dose of 15 mg/kg [26].

Group C (Silymarin Treated): The challenge with DOX was offered to this group through a dose of 5 mg/kg via the intraperitoneal route once weekly for three consecutive weeks to induce toxicity, which makes up a total cumulative dose of 15 mg/kg, simultaneously, Silymarin suspended in 0.5% CMC-Na at a dose of 200 mg/kg was administered daily by oral route over these three weeks [24].

Group D (Vitamin E Treated): Rats were given standard DOX challenge through a dose of 5 mg/kg via the intraperitoneal route once weekly for three consecutive weeks to induce toxicity, which makes up a total cumulative dose of 15 mg/kg, and co-treated with vitamin E suspended in 1.5% HPMC at a dose of 200 mg/kg daily via oral route throughout these three weeks [27].

Group E (Combination Treated): To test possible synergism between antioxidants, rats were given DOX challenge through a dose of 5 mg/kg via the intraperitoneal route once weekly for three consecutive weeks to induce toxicity, which makes up a total cumulative dose of 15 mg/kg, and co-treated with both silymarin (200 mg/kg/day) and vitamin E (200 mg/kg/day) orally during those three weeks.

Group F (natural Recovery): To see how natural healing works, rats in this group got the full DOX doses but without antioxidant treatment, then they were then kept for another three weeks for recovery before they were sacrificed after the last DOX dose.

During the experimental period, a total of three mortalities occurred. One rat in Group C (Silymarin Treated) died prematurely due to an unintended gastrointestinal perforation and subsequent severe necrosis. This was resulted from a technical error during the intraperitoneal administration of doxorubicin. Additionally, two rats in Group F (Natural Recovery) died prior to the conclusion of the six-week period; these deaths were attributed to the severe systemic complications and suspected cardiotoxicity known to be associated with cumulative doxorubicin administration. Consequently, the final effective sample sizes used for all biochemical and histopathological analyses were Group A (n=8), Group B (n=8), Group C (n=7), Group D (n=8), Group E (n=8), and Group F (n=6).

Sample Collection and Preparation

Blood collection: At the start of week 1 baseline blood samples were drawn from all rats to determine initial values, then Terminal blood samples were drawn 24 hours after the end of week 3 for groups A, B, C, D, and E, and 24 hours at the end of week 6 for group F to determine the final values. Once collected, the blood clotted at room temperature, centrifuged (3000 × g for 15 minutes at 4°C) to isolate the serum, and stored at -20°C for later analysis.

Tissue collection: Rats were euthanized, and their livers were removed immediately after collecting the final blood samples. The organs were washed in cold saline to clear excess blood and then divided into two parts. One part was homogenized (10% w/v) in ice-cold phosphate-buffered saline (PBS, 0.1 M, pH 7.4) using a “OMNI BEAD RUPTOR” tissue homogenizer. The homogenates were centrifuged at 13,000 × g for 20 minutes at 4°C using refrigerated centrifuge with trade name “Ouhans” Germany [28], while the other part was

fixed in 10% formalin for histological examination.

To minimize bias in accordance with ARRIVE 2.0 guidelines, all biochemical assays, ELISA measurements, and histopathological tissue analysis were performed by independent investigators who were completely blinded to the animal group allocations.

Biochemical Analysis

Liver damage markers: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) activities, and the total serum bilirubin (TSB) levels were used as primary markers for hepatocellular injury [(29)].

Assessment of Tissue Markers

Oxidative damage was assessed by measuring the levels of malondialdehyde (MDA) and the activities of superoxide dismutase (SOD). These biomarkers act as indicators of the antioxidant defense capacity [(28)].

Tissue responses to injury were assessed by measuring the levels of interleukin-6 (IL-6) and the levels of specific apoptotic markers Caspase-3, Bax, and Bcl-2 [(30)].

Histopathological Examination

The formalin-fixed liver tissue underwent dehydration in graded alcohol and clearing in xylene before being embedded in paraffin wax. slides were cut to a thickness of 5 µm and stained with Hematoxylin and Eosin (H&E). These slides then examined under a light microscope to visualize structural damage such as fatty degeneration, fibrosis, necrosis, and inflammatory cell infiltration.

Statistical Analysis

Data were expressed as mean ± standard error of mean and analyzed using one-way ANOVA then followed by Tukey's post-hoc test to accommodate the unequal sample sizes among the final experimental groups (SPSS v25.0) [(31)]. The graphical presentations were performed using the Python programming language (version 3.10) hosted on the Google Colab platform (Google LLC, Mountain View, CA, USA). P-value less than 5% considered statistically significant.

RESULTS

Liver/Body weight ratio

The ratio in Group B was significantly higher (3.91%) compared to the normal Control (Group A) (3.19%) (Figure 1). This confirms that the liver was enlarged due to DOX-induced hepatotoxicity. In group E both silymarin and vitamin E significantly lowered the ratio to 3.26% ($p < 0.01$). Group D also significantly reduced the ratio to 3.36% ($p < 0.05$). Group C was slightly lower than group B, but the difference was not significant ($p > 0.05$), while group F did not show any improvement. The liver ratio remained high (4.29%) and was not significantly different from group B. The liver ratio remained high (4.29%) and was not significantly different from group B.

Biochemical Analysis

Group B (DOX) showed severe liver injury. DOX caused significant increase in serum AST, ALT, ALP, and TSB levels in group B compared to group A (normal control), as denoted by the distinct significance letters in Figure (2). This confirms the successful induction of liver injury in the experimental model.

Effects on AST and ALP Enzymes

Treatment with silymarin (group C), vitamin E (group D), and their combination (group E) resulted in a significant reduction in both AST and ALP levels when compared to DOX control (Group B), sharing distinct significance letters from Group B (Figure 2a and 2c)., the values in these groups were restored significantly towards normal levels, although they remained slightly elevated compared to the negative control (group A).

Effects on ALT Enzyme

The ALT levels in (Figure 2b) showed groups C, D, E demonstrated a protective effect. Group E showed the most significant reduction compared to group B. Groups C and D also significantly lowered ALT levels relative to group B (denoted by different significance letters in Figure 2b), indicating a mitigation of hepatocellular damage, while group F had no statistical significance reduction in ALT levels and it was comparable to those observed in group B.

Effects on TSB

Total serum bilirubin levels showed different results among groups C, D, E, F in (Figure 2d). Group C and group E showed a significant reduction in TSB levels compared to group B, indicated by their distinct significance letters. Group D did not show a significant reduction and shared the same significance letter as group B. Group F (Natural recovery) showed no statistically significant reduction in TSB levels ($p > 0.05$), which remained comparable to those observed in group B.

Oxidative Stress, Inflammatory, and Apoptotic Markers for Liver Tissue

As shown in (Table 1) group B has marked hepatotoxicity characterized by a significant elevation ($P < 0.001$) in hepatic MDA and IL-6 levels, while SOD activity was significantly reduced in comparison to group A.

Group E showed a strong hepatoprotective effect. The administration of the treatment significantly inhibited the increment in MDA and IL-6 levels, and the SOD activity was restored. The table shows no significant difference between Group E and Group A for these markers.

Group B showed significantly higher up regulation of the pro-apoptotic markers Bax and Caspase-3, and lower levels of Bcl-2.

The Bax/Bcl-2 ratio was significantly elevated in group B (1.52 ± 0.12) compared to group A (0.57 ± 0.01). The Bax/Bcl-2 ratio in group E was (0.61 ± 0.01) close to group A rats.

Histopathological Examination

In (Figure 3), The histological section of rat liver from the negative control (group A) shows intact hepatocytes with organized structure (black arrows), central vein (green arrows), and portal area (blue arrows) at 100X and 400X.

Group B : Photomicrograph of the liver showing severe portal vein congestion (arrows) , slight venular wall thickening, and inflammatory cell infiltration around bile ducts (arrowheads) at 100x, while at 400x the liver parenchyma showing ballooning degeneration of hepatocytes (yellow arrows) and focal area of necrosis (black arrows), with slight sinusoidal congestion.

Group C : Photomicrograph of the liver parenchyma showing mild hepatocyte focal necrosis (black arrow) and mild vacuolar degeneration(yellow arrow) at 100x, while at 400x the liver parenchyma showing hepatocyte mild focal necrosis (arrows) with mild sinusoidal congestion (arrowheads).

Group D : Photomicrograph of the liver parenchyma with mild inflammatory cell infiltration(a) and mild congestion (arrows) at 100x. While at 400x the liver parenchyma with mild inflammatory cell infiltration (arrows).

Group E : Photomicrograph of the liver parenchyma showing normal hepatocytes arrangement with mild congestion(arrows) at 100x, while at 400x the liver parenchyma showing normal hepatocytes arrangement with mild sinusoidal congestion (arrows).

Group F : Photomicrograph of the liver parenchyma shows chronic inflammation represented by moderate sinusoidal dilatation (arrows) at 100x and 400x.

DISCUSSION

The liver weight increased relative to the body weight in group B (DOX) (Figure 1). This is likely due to the fact that doxorubicin causes systemic toxicity (oxidative stress and inflammation), leading to a significant body weight loss at the same time hepatic inflammation could preserve the liver [7,9]. Group E and group D effectively maintained the liver ratio at levels close to group A, showing no statistically significant difference. The protective effect of the combination therapy preserves hepatic structural integrity and prevents drug-induced swelling [6,32]. Group C showed partial protection (marked 'ab') suggests that the combination (Group E) offers a more hepatoprotective effect [14]. Group F shows the highest ratio (marked 'b'), indicating that without antioxidants treatment, hepatic damage and congestion remains even after DOX discontinuation [33,34].

This study validates the hypothesis that the co-administration of silymarin and vitamin E provides superior protection against doxorubicin-induced hepatotoxicity compared to a single agent use or allowing for natural recovery. This cellular damage led to a massive loss of functional integrity of the hepatocyte membrane, as evidenced by the marked elevation of serum AST levels, along with ALT, ALP, and TSB as shown in (Figure 2) , in the DOX-treated group (Group B) ($p < 0.001$) [7,9]. The combination group (Group E) showed the most potent hepatoprotective effect. Both drugs significantly restore serum AST, ALT, and ALP levels towards normal ($p < 0.001$).

Table (1): Effect of treatment on oxidative stress, inflammatory, and apoptotic markers in liver tissue.

Parameters	Group A Mean±SEM	Group B Mean±SEM	Group C Mean±SEM	Group D Mean±SEM	Group E Mean±SEM	Group F Mean±SEM	P-value
MDA (ng/ml)	77.47 ± 0.90 [a] (75.34 – 79.60)	117.10 ±3.72 [d] (108.30– 125.90)	91.00 ± 2.08 [b] (85.91 – 96.09)	101.26 ±1.79 [c] (97.03 – 105.49)	81.95 ± 1.20 [a] (79.11 – 84.79)	108.05 ± 2.84 [cd] (100.75 – 115.35)	< 0.001
SOD (U/ml)	3.58 ± 0.03 [a] (3.51 – 3.65)	2.19 ± 0.15 [c] (1.84 – 2.54)	2.91 ± 0.15 [b] (2.54 – 3.28)	2.75 ± 0.24 [b] (2.18 – 3.32)	3.46 ± 0.08 [a] (3.27 – 3.65)	2.52 ± 0.05 [c] (2.39 – 2.65)	< 0.001
IL-6 (ng/L)	31.75 ± 0.10 [a] (31.51 – 31.99)	49.61 ± 1.71 [d] (45.57 – 53.65)	38.51 ± 1.25 [b] (35.45 – 41.57)	43.94 ± 0.53 [c] (42.69 – 45.19)	33.39 ± 0.49 [a] (32.23 – 34.55)	44.45 ± 1.31 [c] (41.08 – 47.82)	< 0.001
Caspase-3 (ng/ml)	0.78 ± 0.01 [a] (0.76 – 0.80)	1.50 ± 0.13 [d] (1.19 – 1.81)	1.07 ± 0.04 [b] (0.97 – 1.17)	1.21 ± 0.04 [c] (1.12 – 1.30)	0.87 ± 0.02 [a] (0.82 – 0.92)	1.33 ± 0.03 [cd] (1.25 – 1.41)	< 0.001
Bax (ng/ml)	0.91 ± 0.01 [a] (0.89 – 0.93)	1.58 ± 0.13 [d] (1.27 – 1.89)	1.16 ± 0.04 [b] (1.06 – 1.26)	1.36 ± 0.03 [c] (1.29 – 1.43)	1.02 ± 0.02 [a] (0.97 – 1.07)	1.48 ± 0.05 [cd] (1.35 – 1.61)	< 0.001
Bcl-2 (ng/ml)	1.59 ± 0.04 [a] (1.50 – 1.68)	1.05 ± 0.07 [d] (0.88 – 1.22)	1.38 ± 0.05 [b] (1.26 – 1.50)	1.30 ± 0.07 [bc] (1.13 – 1.47)	1.68 ±0.03 [a] (1.61 – 1.75)	1.34 ± 0.09 [b] (1.11 – 1.57)	< 0.001
Bax/Bcl-2 Ratio	0.57 ± 0.01 [a] (0.55 – 0.59)	1.52 ± 0.12 [d] (1.24 – 1.80)	0.84 ± 0.02 [b] (0.79 – 0.89)	1.06 ± 0.04 [c] (0.97 – 1.15)	0.61 ±0.01 [a] (0.59 –0.63)	1.12 ± 0.07 [c] (0.94 – 1.30)	< 0.001

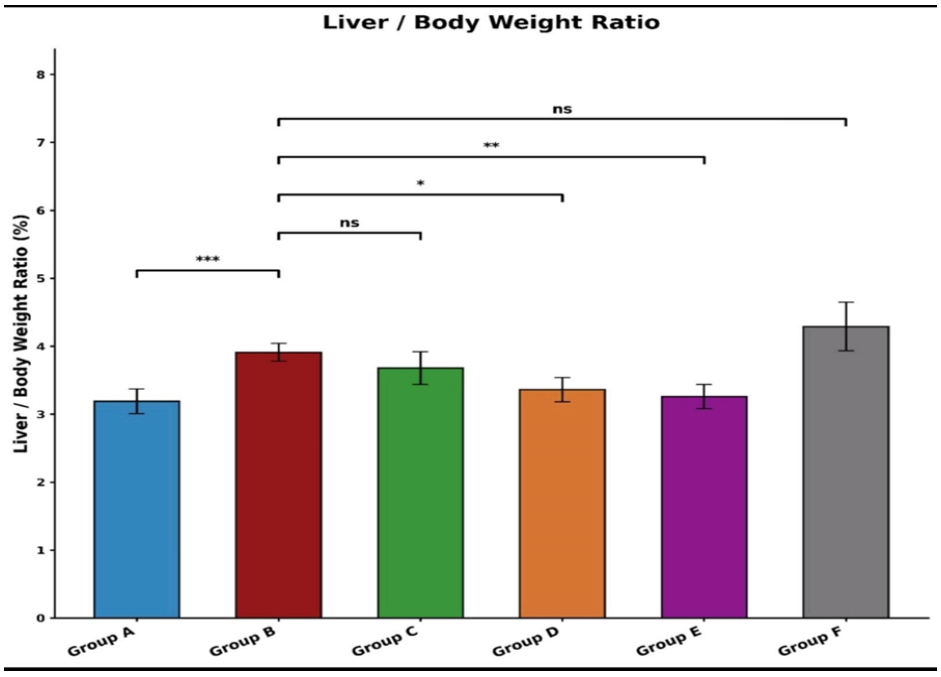


Figure (1) : Effect of treatments on relative liver weight (liver/body weight ratio).

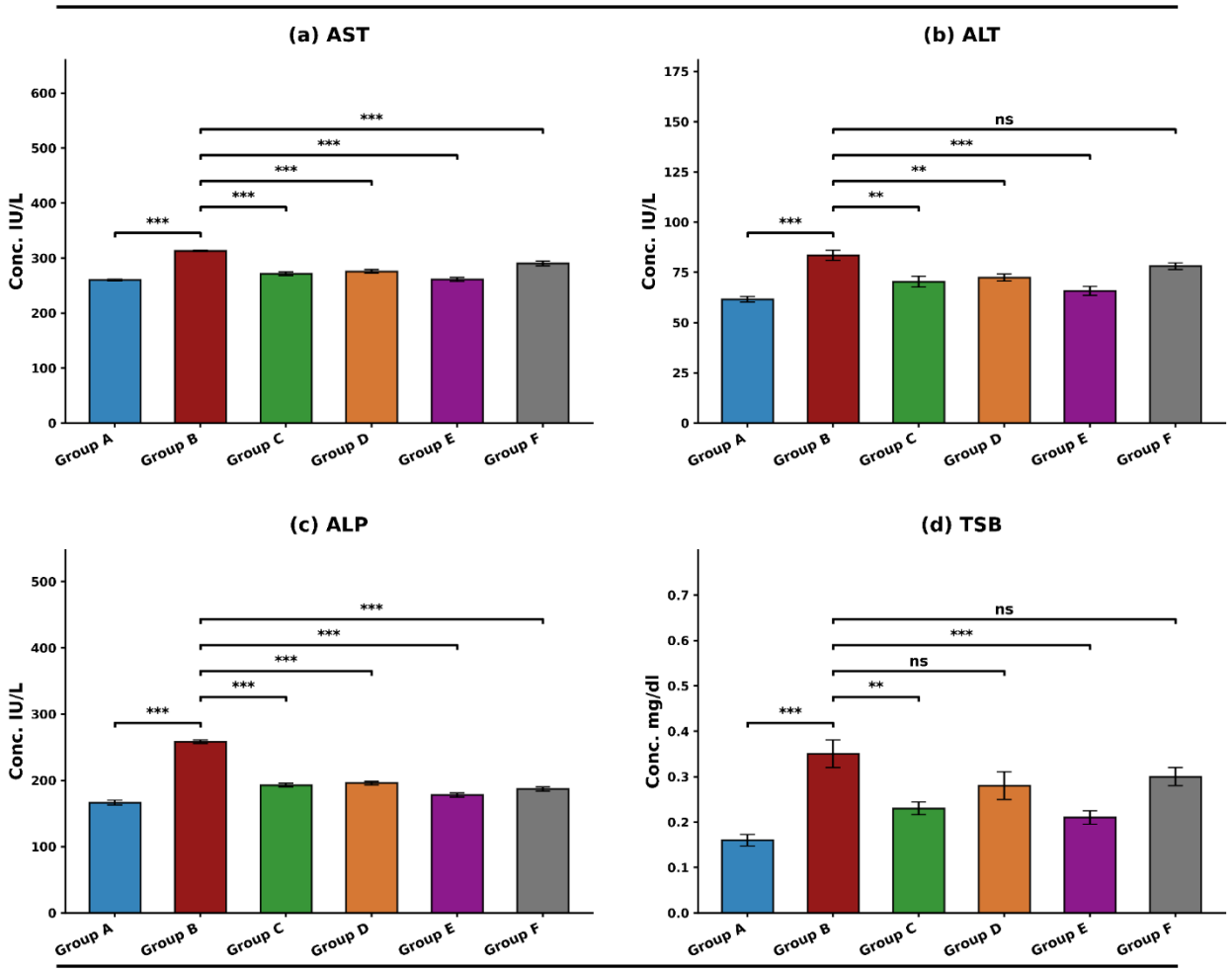
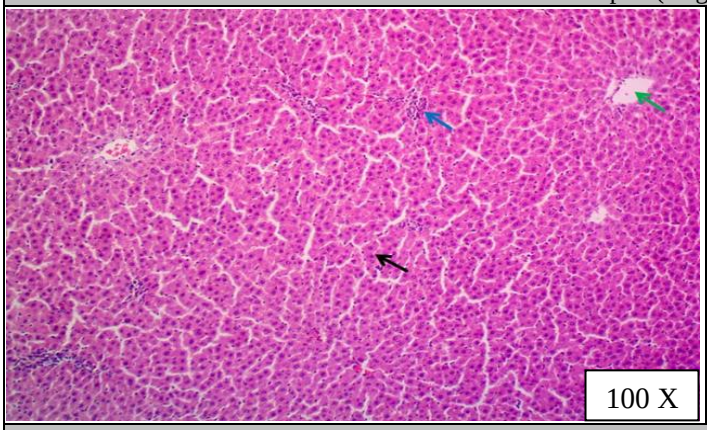
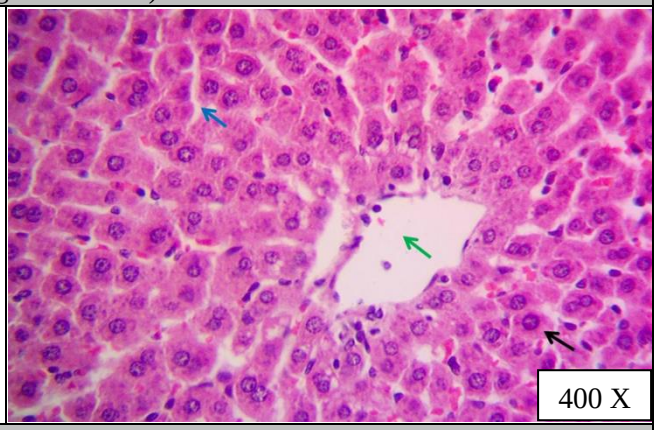


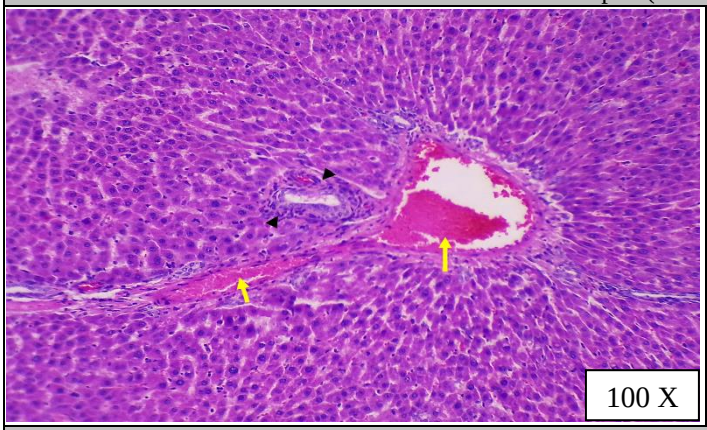
Figure (2) : Effect of treatment on serum biochemical markers of hepatotoxicity in rats.

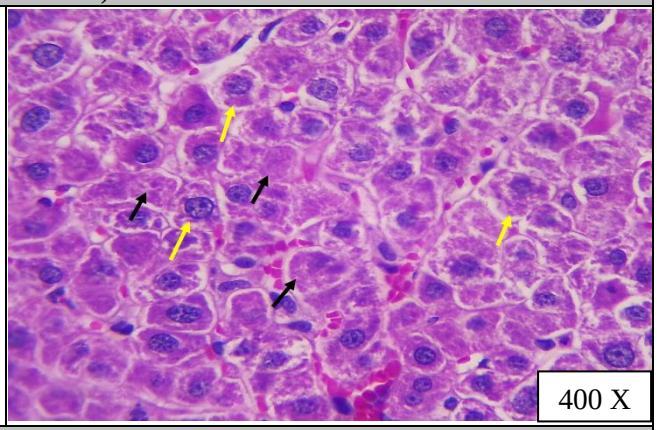
Group A (Negative Control)



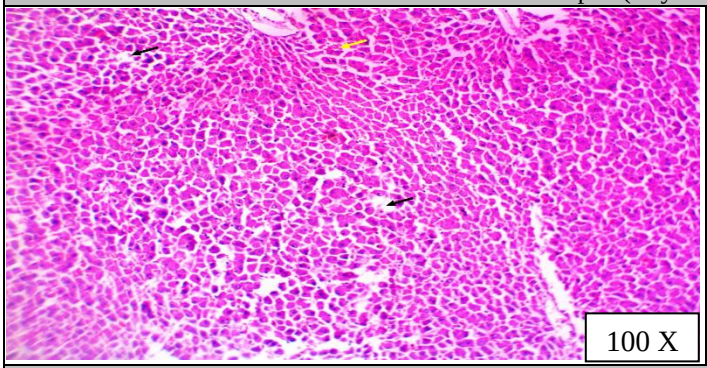


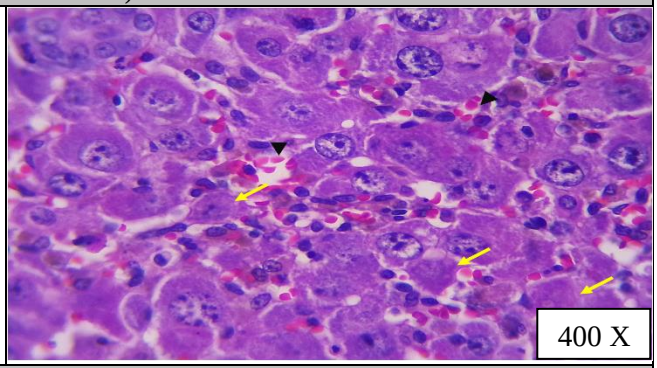
Group B (DOX Control)



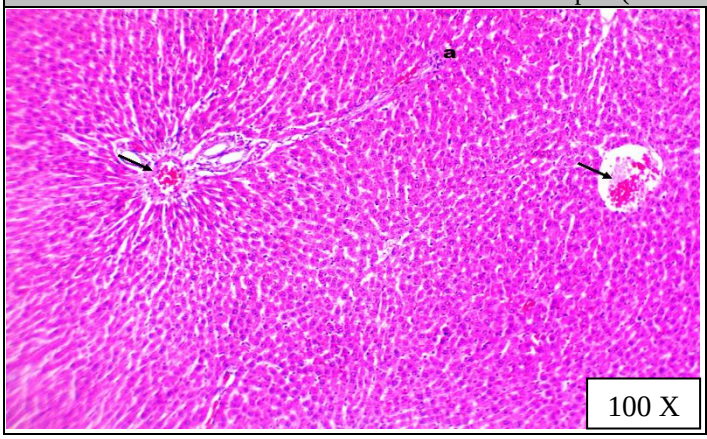


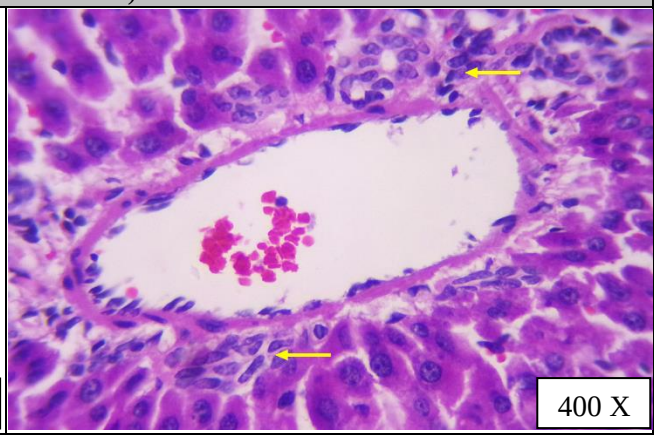
Group C (Silymarin Treated)





Group D (Vitamin E Treated)





(Continued)

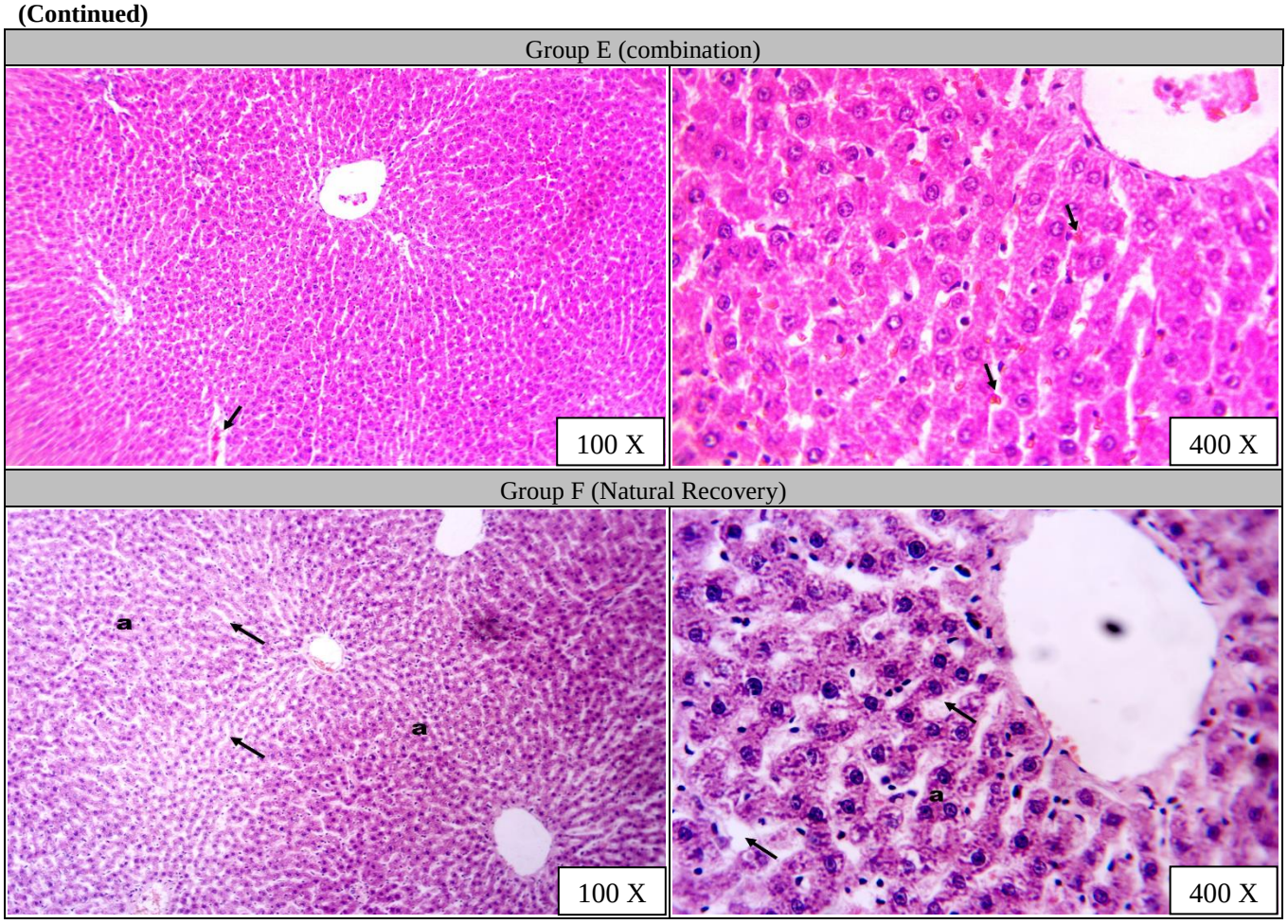


Figure (3): Histopathological changes in hepatic tissue sections stained with hematoxylin and eosin stain across different experimental groups.

Doxorubicin toxicity is largely caused by its metabolism in the liver. The CBR1-mediated reduction generates Reactive Oxygen Species (ROS) that deplete endogenous antioxidants[6,35,36]. In the present study, this oxidative stress was represented by a marked increase in hepatic MDA levels (indicating lipid peroxidation) and a significant decrease in SOD activity shown in (Table 1). Silymarin and vitamin E co-therapy showed a synergistic effect. Silymarin acts as a reducing agent which preserves cellular glutathione (GSH) stores [33,34]. It also gives electrons to regenerate vitamin E and prevent its depletion during oxidative stress [23]. Vitamin E is the primary antioxidant in lipid membranes [16,17,23]. Both agents neutralize reactive metabolites and prevent the lipid peroxidation cycle, as resulted in the significant improvement of MDA levels and SOD activity in the combination group (Group E) [23,32,37]. Furthermore, the study showed the role of inflammation and apoptosis in DOX-induced liver damage. Oxidative stress activates the NF-kappaB pathway, triggering the release of IL-6, which exacerbates tissue damage [15,38]. Doxorubicin also disrupts mitochondrial bioenergetics, triggering the intrinsic apoptotic pathway [20,39,40]. The combination treatment significantly reduces mitochondrial apoptotic signaling. The combination also preserves the Bax/Bcl-2 ratio and reduces Caspase-3 activity, indicating better protection than single agent [13,30].

The histological results of group B showed widespread cell death (necrosis) and blood vessel congestion supporting documented toxicity from oxidative stress and inflammation (Figure 3) [7,38,39]. The natural recovery group (Group F) showed chronic inflammation and congestion indicating that the liver cannot compensate for this damage on its own. Single treatments (silymarin, vitamin E) produced clear reduction in tissue damage [14,17,32]. The most effective outcome was found in the combination group (Group E). The combination treatment preserved normal hepatic structure and provided greater protection than single agents or natural recovery [22,23]. These results suggest that while

chemotherapy disrupts liver metabolism, giving antioxidants can significantly mitigate the injury and preserve hepatic architecture [10,34].

Limitations of The Study : The short duration of the experiment and lack of molecular pathway analysis were the main limitation for this study, additionally the sample size is small so it might not represent the general population. Wistar rats are extensively used in toxicity experiments, but they may produce a response to silymarin or Vitamin E different to that in humans so human model study is greatly suggested to overcome this limitation.

CONCLUSION

Our study concluded that the simultaneous use of silymarin and vitamin E at doses 200mg/kg Orally every day for 3 weeks mitigated doxorubicin-induced hepatotoxicity by normalizing the severe elevation of biochemical, oxidative stress, inflammatory, and apoptotic markers, and severe histological changes induced in liver tissue that induced by doxorubicin at a dose 5mg/kg Intraperitoneally every week for 3 weeks (cumulative dose 15mg/kg).

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AUTHORS CONTRIBUTIONS

Both authors contributed directly and significantly to the study.

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CONFLICTS OF INTEREST

There are no conflicts of interest to declare by the authors about doxorubicin, silymarin, and vitamin E

AVAILABILITY OF DATA AND MATERIAL

Data of this study are available upon request.

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